

Crystalline α -Lactalbumin: An Improved Method for Its Isolation. Sulfur Distribution

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The method recently reported¹ for the isolation of crystalline α -lactalbumin from cows' milk whey has been modified. The present procedure is simpler and gives much better yields.

It has been found that all the sulfur in α -lactalbumin is present as cystine and methionine.

Experimental

Preparation of Crystalline α -Lactalbumin.—Starting with 15 gallons of raw skimmed milk, the procedure previously described¹ is used to remove casein and crude whey globulin, and to crystallize β -lactoglobulin. The clear, yellow supernatant liquid (9 l.) at pH 5.2, from which the β -lactoglobulin crystals were centrifuged, is adjusted to pH 4.0 by the dropwise addition of *N* HCl. A relatively small precipitate, which contains α -lactalbumin, is formed, but it is not removed at this point. Ammonium sulfate (187 g. per liter) is added until a concentration of 1.3 *M* is reached, whereupon more α -lactalbumin is precipitated. The precipitate is centrifuged off, and the supernatant fluid is discarded.² The precipitate is suspended in about 600 ml. of H₂O, and *N* NH₄OH is added dropwise to pH 8.0. Practically all the protein dissolves, although the solution may still be turbid. The solution is clarified by filtration through a thin layer of diatomaceous silica, and the clear filtrate is adjusted to pH 4.0 by the dropwise addition of *N* H₂SO₄, with efficient stirring. The precipitated α -lactalbumin is centrifuged off, and the supernatant fluid is discarded. Crystallization and further purification are carried out as described before,¹ except that reprecipitations are done at pH 4.0 instead of

4.6. The yield of once recrystallized α -lactalbumin is 18.5 g. (anhydrous, salt-free basis). This may be increased by about 20%, as indicated in footnote 2. The yield of 18.5 g. is more than four times that previously reported.¹ Crystalline α -lactalbumin isolated by this procedure is electrophoretically homogeneous at pH 8.5; its mobility at this pH, under the same conditions used before, is -4.2 , a figure identical with that obtained for the original preparation.¹

Sulfur Distribution.—The total cystine-cysteine content of α -lactalbumin was determined in both 6 *N* HCl and HCl-urea hydrolyzates³ by means of the phosphotungstic acid reaction as used by Kassell and Brand.⁴ Slightly higher results ($6.4 \pm 0.1\%$) were obtained with the HCl-urea hydrolyzates than with HCl alone ($6.3 \pm 0.1\%$). Although about 1.5% cysteine was found in the HCl-urea hydrolyzates, it is probable that this was formed as a result of interaction of cystine and tryptophan⁵ (α -lactalbumin contains about 7% tryptophan¹). Sulfhydryl groups in unhydrolyzed α -lactalbumin could not be detected with the nitroprusside test even when the protein was dissolved in 8 *M* guanidine hydrochloride solution. In this respect, α -lactalbumin resembles lysozyme and chymotrypsinogen.⁶

The methionine content of α -lactalbumin was found to be $0.95 \pm 0.05\%$ by the method of Bakay and Toennies.⁶

Thus, the total sulfur of α -lactalbumin, 1.91%,¹ is satisfactorily accounted for in terms of cystine (6.4% cystine = 1.71% S) and methionine (0.95% methionine = 0.20% S).

(2) The supernatant fluid contains additional α -lactalbumin. If maximal yields are sought, the ammonium sulfate concentration is increased to 2.0 *M*, whereupon a second large precipitate separates. This is handled in the same way as the first precipitate at 1.3 *M*. The final yield of α -lactalbumin can be increased by about 20% if this fraction is worked up.

(3) E. Brand and B. Kassell, *J. Gen. Physiol.*, **25**, 167 (1941).

(4) B. Kassell and E. Brand, *J. Biol. Chem.*, **125**, 115 (1938).

(5) H. S. Olcott and H. Fraenkel-Conrat, *ibid.*, **171**, 583 (1947).

(6) B. Bakay and G. Toennies, *ibid.*, **188**, 1 (1951).

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(1) W. G. Gordon and W. F. Semmett, *THIS JOURNAL*, **75**, 328 (1953)